

In the Specification:

Please amend the specification as shown:

Please delete the paragraphs on page ²⁰~~19~~, lines ³⁻¹⁹~~13-29~~ and replace them with the following paragraphs:

Figure 9a is a diagram showing the maps of the plasmids pTip-NH1, pTip-CH1, pTip-LNH1, pTip-LNH1, pTip-NH2, pTip-CH2, pTip-LNH2, and pTip-LCH2. The function of each region and the maps of the plasmids are shown; 6xHis tags disclosed as SEQ ID NO: 168.

Figure 9b shows the DNA sequence of the pTip-NH1 or the pTip-LNH1 from a *TipA* gene promoter sequence or a *TipA-LG10* promoter sequence via a multiple-cloning site to a *ThcA* gene transcription termination sequence; Figure discloses SEQ ID NO: 108 coding SEQ ID NO: 109; short DNA sequence is SEQ ID NO: 110.

Figure 9c shows the DNA sequence of the pTip-CH1 or the pTip-LCH1 from a *TipA* gene promoter sequence or a *TipA-LG10* promoter sequence via a multiple-cloning site to a *ThcA* gene transcription termination sequence; Figure discloses SEQ ID NO: 111 coding SEQ ID NO: 112; short DNA sequence is SEQ ID NO: 113.

Figure 9d shows the DNA sequence of the pTip-NH2 or the pTip-LNH2 from a *TipA* gene promoter sequence or a *TipA-LG10* promoter sequence via a multiple-cloning site to a *ThcA* gene transcription termination sequence; Figure discloses SEQ ID NO: 114 coding SEQ ID NO: 115; short DNA sequence is SEQ ID NO: 116.

Figure 9e shows the DNA sequence of the pTip-CH2 or the pTip-LCH2 from a *TipA* gene promoter sequence or a *TipA-LG10* promoter sequence via a multiple-cloning site to a *ThcA* gene transcription termination sequence; Figure discloses SEQ ID NO: 117 coding SEQ ID NO: 118; short DNA sequence is SEQ ID NO: 119.

Figure 10 is a diagram showing the maps of pTip-CH1.1 (SEQ ID NO: 120), pTip-LCH1.1 (SEQ ID NO: 121), pTip-CH2.1 (SEQ ID NO: 122), and pTip-LCH2.1 (SEQ ID NO: 123);

Please delete the paragraphs on page 20, lines ²⁴⁻²⁸~~7-9~~ and replace it with the following paragraphs:

Figure 12 is a diagram showing a *TipA* gene promoter sequence (SEQ ID NO: 107);

Figure 13 is a diagram showing the modification of a *TipA* gene promoter (SEQ ID NO: 124) to a *TipA-LG10* promoter (SEQ ID NO: 125);

Please delete the paragraphs on page ²¹~~20~~, lines ⁴⁻¹³~~13-21~~ and replace it with the following paragraphs:

Figure 15 is diagram showing the amino acid sequences of five motifs (Motif IV, Motif I, Motif II, Motif III, and C-terminal motif) that are conserved in Rep proteins among pRE8424 (SEQ ID NOS: 126-130), pAP1 (SEQ ID NOS: 131-135), pBL1 (SEQ ID NOS: 136-139), pJV1 (SEQ ID NOS: 140-144), pIJ101 (SEQ ID NO: 145-149), and pSN22 (SEQ ID NO: 150-154). A tyrosine residue allegedly important for the function of the Rep protein is boxed;

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12/15/09

Figure 16 is a diagram showing an especially conserved DNA sequence, of sequences likely to be the DSOs of the pRE8424 (SEQ ID NO: 155), the pAP1 (SEQ ID NO: 156), the pBL1 (SEQ ID NO: 157), the pJV1 (SEQ ID NO: 158), the pIJ101 (SEQ ID NO: 159), and the pSN22 (SEQ ID NO: 160);

Figure 17 is a diagram showing the SSO of the pRE8424 (SEQ ID NO: 161), that is, a sequence of nucleotide Nos. 5268 to 5538 in SEQ ID NO: 90 in the sequence listing, and a possible secondary structure;

Please delete the paragraph on page ²¹~~20~~, line ¹⁶⁻¹⁹~~24-27~~ and replace it with the following paragraph:

Figure 19 is a diagram showing the DNA sequence of *TipA-LG10p-MCS-ALDHt* or *Nit-LG10-MCS-ALDHt*. A wild-type -10 region sequence of a *TipA* gene promoter is CAGCGT, and a -10 region sequence of a *Nit* promoter is TATAAT. These sequences are boxed, respectively; Figure discloses SEQ ID NOS: 162-167, respectively, in order of appearance.

Please delete the paragraph on page ²³⁻²⁷~~21~~, lines ⁴⁻⁸~~4-8~~ and replace it with the following paragraph:

Figure 21 is a photograph showing a result obtained by the following procedures: *PIP* and *GFP* genes are incorporated into two vectors that do not cause incompatibility with each other, *PIP* and *GFP* expressed in a single *R. erythropolis* strain JCM3201 cell are purified and analyzed by SDS polyacrylamide electrophoresis, followed by the staining of the gels with Coomassie Brilliant Green G-250. 6xHis tags disclosed as SEQ ID NO: 168.

Please delete the paragraph on page ³⁰~~29~~, lines ⁹⁻²¹~~15-27~~ and replace it with the following paragraph:

Next, primers represented by SEQ ID NOs: 23 and 24 in the sequence listing were used to perform amplification by PCR with a plasmid pRSET-PIP (Tamura et al., FEBS Lett. 398 101-105 [1996]; hereinafter, abbreviated to PIP) as a template. The primer represented by SEQ ID NO: 24 in the sequence listing is designed so that 6xHis tag (SEQ ID NO: 168) is attached to the C terminus of a PIP protein in order to eliminate the termination codon of the *PIP* gene and facilitate protein purification. The 6xHis tag (SEQ ID NO: 168) is a consecutive sequence consisting of six consecutive histidine residues, and a protein fused with this tag exhibits high affinity for a nickel ion or the like. Thus, the protein is readily purified by metal chelate chromatography that employs the nickel ion or the like (Crowe et al., Methods Mol. Biol. 31 371-387 [1994]). This 0.9-kb DNA fragment containing the *PIP* gene was doubly digested with restriction enzymes *Ncd* and *Spd* and subcloned into the *Ncd* and *Spd* sites of the pHN150u. Consequently, a plasmid containing the ORF of the *PIP* gene located immediately downstream of the *TipA* gene promoter sequence was constructed and designated as pHN151u.

Please delete the paragraph on page ⁴⁶~~45~~, lines ¹⁴⁻²⁵~~9-20~~ and replace it with the following paragraph:

Although both of the pHN171 and the pHN348 were expression vectors where a *PIP* gene, a reporter gene, was introduced into the MCS of the pTip vector (see Reference Example), the difference between them is only in a transformation marker: a tetracycline resistance gene for the pHN171 and a chloramphenicol resistance gene for the pHN348. In any of the plasmids, a ribosome-binding site sequence originally located downstream of the *TipA* gene promoter (*TipA*-RBS) is altered into a bacteriophage *gene 10*-derived ribosome-binding site sequence having good translation efficiency (*TipA-LG10* promoter; see Reference Example). A 6xHis tag (SEQ ID NO: 168) is adapted to be attached to the C terminus of PIP in order to facilitate protein purification. The 6xHis tag (SEQ ID NO: 168) is a consecutive sequence consisting of six consecutive histidine residues, and a protein fused with this tag exhibits high affinity for a nickel ion or the like. Thus, the protein is readily purified by metal chelate chromatography that employs the nickel ion or the like (Crowe et al., Methods Mol. Biol. 31 371-387 [1994]).

Please delete the paragraphs on page ⁵⁵~~54~~, line ¹⁸~~7~~ to page ⁵⁹~~55~~, line ²~~16~~ and replace them with the following paragraphs:

At first, primers represented by SEQ ID NO: 86 (sHN337) and 87 (sHN338) in the sequence listing were used to perform DNA amplification by PCR with the pHN187 (see Reference Example 1) as a template. The obtained 0.2-kb fragment contains the 5' end portion of the *GFP* gene. This fragment was digested with *Ncd*, and its 5' ends were phosphorylated. On the other hand, primers represented by SEQ ID NO: 88

polyacrylamide electrophoresis according to an ordinary method. A result of staining the gel with Coomassie Brilliant Green G-250 after analysis by SDS polyacrylamide electrophoresis is shown in Figure 21.

Please delete the Table 1 header on page ⁵⁸~~57~~ and replace it with the following header:



12/15/09

Table 1

Table 1 Main plasmids used in the present invention

(6xHis tags disclosed as SEQ ID NO: 168)

Amendments to the Specification

Please replace paragraph [0018] at page ⁵ 2 of the published patent application with the following rewritten paragraph:

[1] DNA comprising a nucleotide sequence of a mutated TipA gene promoter where a mutation is introduced into a -10 region sequence of a TipA gene promoter, the mutated TipA gene promoter capable of thiostrepton-independent and constitutive expression of a gene located downstream thereof;

[2] The DNA of [1], wherein the mutation in the -10 region sequence is a mutation of a CAGCGT sequence to a TATAAT sequence;

[3] The DNA of [2], having a nucleotide sequence represented by ~~SEQ ID NO: 107~~; **SEQ ID NO: 169 or SEQ ID NO: 170.**

Please replace paragraph [0045] at page ¹² 8 of the published patent application with the following rewritten paragraph:

The present invention further encompasses an expression vector comprising a Rep gene, a double-stranded origin (DSO), and a single-stranded origin (SSO) as a DNA region necessary for the rolling circle mode of replication obtained from the plasmid and further comprising a promoter sequence, a ribosome-binding site sequence located downstream of the promoter sequence, and a multiple-cloning site sequence capable of incorporating a foreign gene therein, located downstream of the ribosome-binding site sequence. The expression vector may further contain a foreign gene and a transcription termination sequence. The DNA sequence having promoter activity, the foreign gene, and the transcription termination sequence constitute an expression cassette. The promoter sequence used here includes a promoter capable of inducer (such as a drug)-inducible expression of a foreign gene introduced downstream thereof and a promoter capable of inducer-independent and constitutive expression of a foreign gene. Examples of the former promoter capable of inducible expression of a foreign gene include a TipA gene promoter that inducibly expresses a foreign gene located downstream thereof in the presence of thiostrepton. The vector of the present invention may

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12/15/09

comprise a TipA gene encoding a TipA protein and an appropriate promoter inducing the expression of the TipA gene, such as a ThcA gene promoter. The TipA gene and the promoter for the expression of the TipA gene constitute an inducer cassette. When a host cell is a bacterium belonging to the genus Rhodococcus, a thiostrepton resistance gene or the like that imparts resistance to thiostrepton is incorporated into the vector because the bacterium is sensitive to thiostrepton. In addition, the TipA gene promoter may be any of those obtained by modifying the sequence of the TipA gene promoter, such as a TipA-LG10 promoter. The sequence of a mutant the TipA gene promoter is shown as SEQ ID NO: 170 in FIG. 12.

Please replace paragraph [0072] at page ²⁰~~8~~ of the published patent application with the following rewritten paragraph:

FIG. 12 is a diagram showing a mutant TipA gene promoter sequence (~~SEQ ID NO: 107~~) (SEQ ID NO: 170);

Please replace paragraph [0123] at page ³⁴~~12~~ of the published patent application with the following rewritten paragraph:

Primers represented by SEQ ID NOs: 21 and 37 in the sequence listing were used to perform amplification by PCR with the plasmid pHN170 as a template. As a result, a hybrid promoter (hereinafter, indicated by a TipA-LG10 promoter; indicated by TipA-LG10p in the drawings) consisting of the TipA gene promoter and the ribosome-binding site derived from the lambda phage gene 10 was obtained. This 0.2-kb DNA fragment was doubly digested with restriction enzymes BsrGI and NcoI and subcloned into the BsrGI and NcoI sites of the pHN170. Consequently, a plasmid containing the PIP gene placed under the control of the TipA-LG10 promoter was constructed and designated as pHN171. FIG. 12 shows ~~the~~ a mutant TipA promoter sequence (SEQ ID NO: 170), and FIG. 13 shows the modification of the ribosome-binding site (RBS) sequence for altering the mutant TipA promoter (SEQ ID NO: 170) into the TipA-LG10 promoter (SEQ ID NO: 169).